13-14. This region undergoes significant conformational changes during sugar translocation. Moreover, the results from the thio-D-glucose coupled via a long heterobifunctional crosslinker with a small end group showed the possibility to observe the D-glucose transport pathway of the SGLT1. These studies demonstrate that AFM is a powerful method to explore the structural and functional dynamics of plasma membrane transport proteins in live cells on a single molecule level.

979-Pos

Simultaneous Topography and Recognition (TREC) of Proteins in the Pathological Deposits in Pseudoexfoliation Syndrome using AFM

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Protein aggregation is of significant interest to various disciplines; it can be the cause of debilitating diseases, or the foundation of advanced nanomaterials. One ocular disease hallmarked by protein aggregation is known as Pseudoexfoliation Syndrome (PEX). This condition is caused by the formation of insoluble aggregates, and is characterised by deposition of fibrillar proteinaceous material on the anterior lens capsule. PEX deposits in the eye block the aqueous outflow mechanisms, which can lead to an elevation in intraocular pressure and subsequent glaucoma. Glaucoma is the second leading cause of irreversible blindness worldwide, and PEX is the most common known risk factor for glaucoma.

Proteomic analyses have revealed an association of various genetic markers and protein expression with PEX; however a complete explanation for disease susceptibility is not known. As the aggregates are a complex arrangement of proteins, the ultrastructure is poorly characterised and many protein constituents of the aggregates remain unknown. This study addresses the critical issue of determining the molecular nature of PEX on lens capsules in their native state by atomic force microscopy (AFM) based antibody recognition imaging. This AFM methodology is referred to as Topography and RECognition imaging (TREC). Proteins identified as being implicated in the PEX pathophysiology are detected by an AFM probe modified with the appropriate antibody. Topographical AFM images and antibody recognition images are obtained simultaneously to determine the specific location of proteins in and around PEX aggregates. This data, combined with data from proteomic and genetic analyses, is leading to an improved understanding of the pathophysiological basis of PEX. A more complete understanding of the pathophysiological basis for the disease will lead to earlier detection methods and treatments that target the disease instead of the subsequent glaucoma.

980-Pos

Deciphering Podosome Physical Properties in Human Macrophage by Atomic Force Microscopy

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Macrophages belong to phagocytes which constitute the first line of host defense. Directed migration and adhesion of these cells through anatomic boundaries is crucial for their functions. Moreover, tissue infiltration of macrophages has been shown to worsen many pathologies such as atherosclerosis, chronic inflammation and cancer. Our research focuses on understanding the adhesive and motile behaviors of macrophages with particular emphasis on podosomes, dynamic actin-rich structures only found in macrophage and macrophage-derived cells required for normal adhesion and migration. Although the minimal structural feature of podosome can be defined as an F-Actin-rich core surrounded by a ring containing proteins such as Vinculin and Integrins, the mechanisms involved in podosome biogenesis and architecture are poorly understood. Thanks to the atomic force microscope operated in liquid, it is now possible to explore cells at the nanoscale in terms of topographic and force measurement mode. Here we choose to use this powerful technique to explore the mechanisms involved in biogenesis, bio-physical properties and architecture of podosome in human monocyte-derived macrophage. Micro-contact printing technique was used to generate patterns of different physiological extra-cellular matrix (ECM) proteins in order to delineate podosome formation in vitro and investigate the influence of the nature of the substrate on their bio-physical properties knowing that the molecular recognition of ECM protein inducing podosome formation involved an integrin-dependant signaling. Our preliminary experiments using AFM allowed us to measure the height, the dynamic and the Young's modulus of podosomes in different situations. We will present our last results of this ongoing work.

981-Pos

Geometric Influences on Radial Indentation of Microtubules

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Microtubules assembled in vitro exist in a variety of configurations that vary in number of protofilaments, radius, and skew angle of protofilaments relative to the main microtubule axis. Such variations affect microtubule stability, energetics, and assembly/disassembly dynamics. Further, the most abundant microtubule geometries observed in vitro are influenced by assembly conditions and stabilization methods. We have studied the relationship between microtubule geometry and mechanical properties using finite element modeling (FEM). Specifically, we have examined the effects of protofilament number, microtubule radius, and protofilament skew on the radial stiffness (effective radial spring constant of the microtubule wall) of microtubules as measured in atomic force microscopy (AFM) experiments. Our previous AFM work determined that microtubules assembled in the presence of a slowly-hydrolysable GTP analog, GMPCPP, have enhanced radial stiffness relative to those stabilized with paclitaxel. We surmise that in vitro populations of GMPCPP-microtubules and paclitaxel microtubules contain distinct distributions of microtubule geometries, so we have used FEM to examine the relative effect of microtubule geometry on stiffness values we measure. Our modeling results indicate that the changes in stiffness that we have observed experimentally are not simply a result of changes in protofilament number or orientation but instead are likely due to a relative change in material properties (e.g. effective Young's modulus) of the tubulin polymers.

982-Pos

Organization of RAG1/2 and RSS DNA in the Post-Cleavage Complex

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V(D)J recombination is central to establishing a functional adaptive immune system. The large repertoire of immunoglobulins and T-cell receptors is generated by combinatorial rearrangement of an extensive array of variable (V), diversity (D), and joining (J) gene segments that are joined to encode the variable domains of the protein chains. The recombination signal sequences (RSS) that flank these gene segments are recognized, paired in a synaptic complex, and cleaved by collaboration of the lymphoid-specific proteins RAG1 and RAG2. After cleavage, the signal ends remain tightly bound to the RAG proteins in a particularly stable Signal-End Complex (SEC).

To obtain 3D structural information about RAG1/2 bound to RSS DNA, isolated and purified SEC were visualized by AFM. To better define the arrangement of the RAG proteins and RSS DNA in the complex, we used RAG1 and RAG2 fused with maltose binding protein (MBP). A wide variety of complex shapes was recorded, however, it was clear that the two DNA chains predominantly exited the SEC complex from adjacent points. The volume of the protein core was consistent with the expected mass of 500 kDa corresponding to (RAG1)2-(RAG2)2 composition. MBP protrusions could be observed on the protein particles marking the N-termini of RAG1 and RAG2. To make their appearance more noticeable, we used selective antibody labeling. Fab-labeled MBPs were clearly identified peripheral to the recombinase core. When only the RAG2 MBPs were labeled, the two DNAs most often exited together from the SEC on the opposite side to the Fabs. Consistent with this observation, when only the RAG1 MBPs were labeled, they were situated closer to the exiting DNAs.

The parallel arrangement of DNA and protein subunits found by AFM is in an excellent agreement with the 3D model based on EM data.

983-Pos

Nanoscale Tissue Scaffold Investigations to Optimize Central Nervous System Prosthetic

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The introduction of scaffolding materials with appropriate biochemical cues and physical properties into damaged sites within the central nervous system can encourage endogenous or exogenous cellular re-colonization. The scaffolding material currently under investigation is a synthetic electrospun polyamide

nanofibrillar matrix that has demonstrated promise in tissue engineering approaches for the repair of the injured spinal cord and is architecturally mimetic for the capillary basement membrane at the blood brain barrier. We will present quantitative investigations of the nanofibrillar matrix, which are achieved through use of a dynamic new mode of atomic force microscopy, Scanning Probe Recognition Microscopy (SPRM). SPRM uniquely allows auto-tracking along individual nanofibers, which are then compiled into a statistical representation of the nanofibrillar matrix as a whole. Complementary transmission electron microscopy (TEM) and nuclear magnetic resonance (NMR) investigations are performed to assess nanofiber internal structures that contribute to elasticity, and growth factor binding site information, respectively. Results of investigations of unmodified nanofibers and nanofibers covalently modified with fibroblast growth factor-2 (FGF-2), a prevailing cytokine involved in regulation of the growth of astrocytes, neurons, and other neural cells, will be presented. Astrocytes are neural cells that can be considered to be the cellular bridge between the capillary basement membrane and neurons. They are therefore directly responsive to the biochemical cues and physical properties of the native or prosthetic basement membrane. We will present results achieved through SPRM, immunocytochemistry, and Western blot techniques that indicate that there are significant differences in the astrocyte response to 2D planar substrates versus 3D nanofibrillar substrates versus 3D nanofibrillar substrates that are covalently modified with FGF-2, mimicking the sequestration of growth factors on the basement membrane. Differences in astrocyte physiology, substrate probing through lamellipodia and filopodia extension and FGF-2 up-regulation will be discussed.

984-Pos

Misfolding and Aggregation of Amyloid Beta Peptide: Single Molecule AFM Force Spectroscopy

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Misfolding and aggregation of amyloid beta (Aβ) peptide result in development of Alzheimer's disease, and Aβ dimers are considered as the smallest neurotoxic species. The aggregates formed by Aβ-peptides have been characterized by various techniques, but our knowledge on the molecular mechanism underlying the processes of misfolding and the early stages of aggregation of the peptides is limited. We have shown earlier that AFM force spectroscopy is capable of detecting protein misfolded states and characterizing the initial stages of the protein aggregation. Importantly, we showed that α-synuclein dimers are stable transient states playing role of triggers in the process of the protein self-assembly in nanopartiles and fibrils. Here we applied the sample methodology to probing and characterizing of misfolding of Aβ40 peptide. The protein was immobilized on the AFM tip and the surface and the interaction between the proteins was measured in multiple approach-retraction cycles. Using this approach was able to analyze interprotein interactions at single molecule level. The force spectroscopy analysis provided us with the following important information. First, using Dynamic Force Spectroscopy (DFS) approach we characterized pathways of Aβ40 misfolding. The lifetimes of transient Aβ40 dimers can be as long as several seconds suggesting that formation of the states with such a lifetimes can trigger the aggregation. Second, the pathways for the misfolding and aggregation depend on pH leading to a rather complex energy landscape reconstructed from the DFS data. Third, the analysis of the contour lengths supported the conclusions on various aggregation pathways and led to the models for such conformations of Aβ40.

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985-Pos

Nanowires As AFM Cantilevers: A Detection Scheme to Gently Image Soft Biological Materials in Fluids

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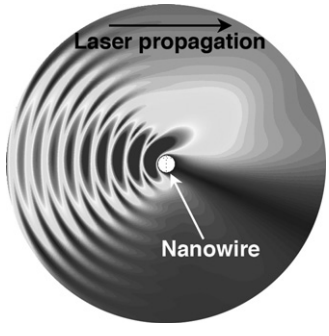
Performing AFM on soft materials in fluids (e.g., living cells) is challenging due to their ready deformation by the tip. The thermal force-noise of the cantilever is the principal limitation to reducing sample deformation and minimizing a cantilever's cross- section reduces its noise significantly. However, the minimum size of the cantilever is currently limited by a conventional deflection detection scheme, which requires a large surface area for laser specular reflection. Here we develop an optical technique to use nanowires as cantilevers, and show that we achieve a force noise in water that is orders of magnitude gentler than conventional AFM. This is a significant milestone towards non-invasive scanning probe imaging of biological processes on the surfaces of vesicles and cell membranes.



986-Pos

Nucleosome Dynamics : Atomic Force Microscopy Reveals its Intimity

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Recent genome-wide nucleosome mappings along with bioinformatics studies have confirmed that the DNA sequence plays a more important role in the collective organization of nucleosomes in vivo than previously thought. Yet, in living cells, this organization of nucleosomes also results from the action of various external factors like DNA binding proteins and chromatin remodelers. To decipher the code for intrinsic chromatin organization and dynamics, there is thus a need for in vitro experiments to bridge the gap between computational models of nucleosome sequence preferences and in vivo nucleosome occupancy data. Here we first combine atomic force microscopy (AFM) in liquid and theoretical modeling to demonstrate that the main sequence signaling in vivo are high energy barriers that locally inhibit nucleosome formation rather than favourable positioning motifs. We show that these excluding genomic energy barriers condition the collective assembly of neighboring nucleosomes consistently with equilibrium statistical ordering principles. The analysis of two gene promoter regions in S.cerevisiae and the human genome indicates that these genomic barriers direct the intrinsic nucleosome occupancy of regulatory sites, thereby contributing to gene regulation. We further apply time-lapse AFM imaging to directly visualize the dynamics of a single nucleosome nearby a genomic excluding energy barrier. The observation, in the absence of remodelers, of the unwrapping and/or ejection of this nucleosome suggests that the sequence-dependent intrinsic nucleosome dynamics can contribute to chromatin remodeling. These results provide novel hypotheses about chromatin dynamics and its contribution to gene regulation.

987-Pos

Mechanically Induced Cell Signaling Stimulates Real-Time Cytoskeleton Remodeling

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External mechanical stresses alter the structural and functional properties of the cells, leading to rapid responses that induce adaptive changes to the external environment. The extracellular matrix is responsible for a complex cross-talk needed for transmitting environmental signals to the cell through the focal adhesions as mediators of the process. An Atomic Force Microscope (AFM) probe functionalized with fibronectin was able to mechanically stimulate the apical surface of a live smooth muscle cell inducing significant changes in cell shape that can be recorded in real time by optical imaging. Due to the strong focal adhesion formed around the AFM tip, the cytoskeletal elements are directly manipulated through a matrix-integrin-actin linkage between the cell and the fibronectin coated tip. Following each controlled upward movement of the cantilever, the cell responds by presenting a biphasic change in height dependent of the treatment applied, and independent of time. In the same time, the cell reinforces its attachment to the substrate to better resist the mechanical stimulation by increasing focal adhesion and actin area at the basal cell level. Our measurements showed significant differences between control cells and cells where the intracellular tension was modulated by RhoA. Thus, in cells transfected with RhoA constitutively active the cell reactive-response presents higher amplitude than control because the cell is stronger due to the presence of more actin fibers. A different response was found when cells were transfected with RhoA dominant negative, which decreases intracellular tension, such that actin filaments are present only at the cell boundaries. Under these conditions, at the same force level, the AFM tip detaches from the apical cell surface. These innovative approaches offer new information for understanding live cell remodeling and dynamics in response to mechanical force.

988-Pos

AFM and SMFS of Clathrin Triskelia under Fluid

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